

## **MUSCARINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS**

### **FIELD OF THE INVENTION**

This invention relates to novel derivatives of 8-azoniabicyclo[3,2,1]octanes, pharmaceutical compositions, processes for their preparation, and use thereof in  
5 treating M<sub>3</sub> muscarinic acetylcholine receptor mediated diseases.

### **BACKGROUND OF THE INVENTION**

Acetylcholine released from cholinergic neurons in the peripheral and central nervous systems affects many different biological processes through interaction with two major classes of acetylcholine receptors – the nicotinic and the muscarinic  
10 acetylcholine receptors. Muscarinic acetylcholine receptors (mAChRs) belong to the superfamily of G-protein coupled receptors that have seven transmembrane domains. There are five subtypes of mAChRs, termed M<sub>1</sub>-M<sub>5</sub>, and each is the product of a distinct gene. Each of these five subtypes displays unique pharmacological properties. Muscarinic acetylcholine receptors are widely distributed in vertebrate organs, and  
15 these receptors can mediate both inhibitory and excitatory actions. For example, in smooth muscle found in the airways, bladder and gastrointestinal tract, M<sub>3</sub> mAChRs mediate contractile responses. For review, please see {Brown 1989 247 /id}.

Muscarinic acetylcholine receptor dysfunction has been noted in a variety of different pathophysiological states. For instance, in asthma and chronic obstructive  
20 pulmonary disease (COPD), inflammatory conditions lead to loss of inhibitory M<sub>2</sub> muscarinic acetylcholine autoreceptor function on parasympathetic nerves supplying the pulmonary smooth muscle, causing increased acetylcholine release following vagal nerve stimulation. This mAChR dysfunction results in airway hyperreactivity mediated by increased stimulation of M<sub>3</sub> mAChRs {Costello, Evans, et al. 1999 72 /id} {Minette, Lammers, et al. 1989 248 /id}. Similarly, inflammation of the gastrointestinal tract in  
25 inflammatory bowel disease (IBD) results in M<sub>3</sub> mAChR-mediated hypermotility {Oprins, Meijer, et al. 2000 245 /id}. Incontinence due to bladder hypercontractility has also been demonstrated to be mediated through increased stimulation of M<sub>3</sub> mAChRs {Hegde & Eglen 1999 251 /id}. Thus the identification of subtype-selective  
30 mAChR antagonists may be useful as therapeutics in these mAChR-mediated diseases.

Despite the large body of evidence supporting the use of anti-muscarinic receptor therapy for treatment of a variety of disease states, relatively few anti-

muscarinic compounds are in use in the clinic. Thus, there remains a need for novel compounds that are capable of causing blockade at M<sub>3</sub> mAChRs. Conditions associated with an increase in stimulation of M<sub>3</sub> mAChRs, such as asthma, COPD, IBD and urinary incontinence would benefit by compounds that are inhibitors of mAChR binding.

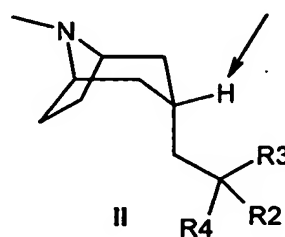
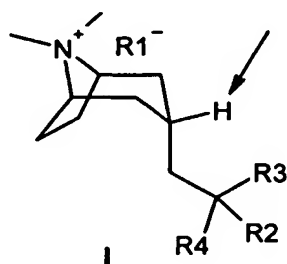
### SUMMARY OF THE INVENTION

This invention provides for a method of treating a muscarinic acetylcholine receptor (mAChR) mediated disease, wherein acetylcholine binds to an M<sub>3</sub> mAChR and which method comprises administering an effective amount of a compound of Formula (I) or Formula (II) [except the compound of Formula (II) with R<sub>2</sub> and R<sub>3</sub> as 2-thiophene and R<sub>4</sub> as -OC(O)CH<sub>3</sub>] or a pharmaceutically acceptable salt thereof.

This invention also relates to a method of inhibiting the binding of acetylcholine to its receptors in a mammal in need thereof which comprises administering to aforementioned mammal an effective amount of a compound of Formula (I) or Formula (II).

The present invention also provides for the novel compounds of Formula (I) or Formula (II), and pharmaceutical compositions comprising a compound of Formula (I) or Formula (II), and a pharmaceutical carrier or diluent.

Compounds of Formula (I) or Formula (II) useful in the present invention are represented by the structure:



wherein:  
the H atom indicated is in the exo position;

R1<sup>-</sup> represents an anion associated with the positive charge of the N atom. R1<sup>-</sup> may be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate and toluene sulfonate;

- 5 R2 and R3 are independently selected from the group consisting of straight or branched chain lower alkyl groups (having preferably from 1 to 6 carbon atoms), cycloalkyl groups (having from 5 to 6 carbon atoms), cycloalkyl-alkyl (having 6 to 10 carbon atoms), heterocycloalkyl (having 5 to 6 carbon atoms) and N or O as the heteroatom, heterocycloalkyl-alkyl (having 6 to 10 carbon atoms) and N or O as the heteroatom,  
 10 aryl, optionally substituted aryl, heteroaryl, and optionally substituted heteroaryl;

- R4 is selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, aryl, heteroaryl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-aryl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-heteroaryl, -OR<sub>5</sub>,  
 15 -CH<sub>2</sub>OR<sub>5</sub>, -CH<sub>2</sub>OH, -CN, -CF<sub>3</sub>, -CH<sub>2</sub>O(CO)R<sub>6</sub>, -CO<sub>2</sub>R<sub>7</sub>, -CH<sub>2</sub>NH<sub>2</sub>, -CH<sub>2</sub>N(R<sub>7</sub>)SO<sub>2</sub>R<sub>5</sub>, -SO<sub>2</sub>N(R<sub>7</sub>)(R<sub>8</sub>), -CON(R<sub>7</sub>)(R<sub>8</sub>), -CH<sub>2</sub>N(R<sub>8</sub>)CO(R<sub>6</sub>), -CH<sub>2</sub>N(R<sub>8</sub>)SO<sub>2</sub>(R<sub>6</sub>), -CH<sub>2</sub>N(R<sub>8</sub>)CO<sub>2</sub>(R<sub>5</sub>), -CH<sub>2</sub>N(R<sub>8</sub>)CONH(R<sub>7</sub>);

- R5 is selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-aryl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-heteroaryl;  
 20

- R6 is selected from the group consisting of (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, aryl, heteroaryl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-aryl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-heteroaryl;  
 25

- R7 and R8 are, independently, selected from the group consisting of H, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>12</sub>)cycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl(C<sub>3</sub>-C<sub>7</sub>)heterocycloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkyl-aryl, and (C<sub>1</sub>-C<sub>6</sub>)alkyl-heteroaryl.  
 30

Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonc acid, ethane sulphonc acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of Formula (I) or Formula (II) may also be formed with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

The following terms, as used herein, refer to:

- "halo" - all halogens, that is chloro, fluoro, bromo and iodo.
- "C<sub>1-10</sub>alkyl" or "alkyl" - both straight and branched chain moieties of 1 to 10 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl and the like.
- "cycloalkyl" is used herein to mean cyclic moiety, preferably of 3 to 8 carbons, including but not limited to cyclopropyl, cyclopentyl, cyclohexyl, and the like.
- "alkenyl" is used herein at all occurrences to mean straight or branched chain moiety of 2-10 carbon atoms, unless the chain length is limited thereto, including, but not limited to ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl and the like.
- "aryl" - phenyl and naphthyl;
- "heteroaryl" (on its own or in any combination, such as "heteroaryloxy", or "heteroaryl alkyl") - a 5-10 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, pyrazole, furan, thiophene, quinoline, isoquinoline, quinazolinyl, pyridine, pyrimidine, oxazole, tetrazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole.
- "heterocyclic" (on its own or in any combination, such as "heterocyclicalkyl") - a saturated or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, or S; such as, but not limited to, pyrrolidine, piperidine, piperazine, morpholine,

tetrahydropyran, thiomorpholine, or imidazolidine. Furthermore, sulfur may be optionally oxidized to the sulfone or the sulfoxide.

• "arylalkyl" or "heteroarylalkyl" or "heterocyclicalkyl" is used herein to mean C<sub>1-10</sub> alkyl, as defined above, attached to an aryl, heteroaryl or heterocyclic moiety, as  
5 also defined herein, unless otherwise indicated.

• "sulfinyl" - the oxide S (O) of the corresponding sulfide, the term "thio" refers to the sulfide, and the term "sulfonyl" refers to the fully oxidized S(O)<sub>2</sub> moiety.

• "wherein two R<sub>1</sub> moieties (or two Y moieties) may together form a 5 or 6  
10 membered saturated or unsaturated ring" is used herein to mean the formation of an aromatic ring system, such as naphthalene, or is a phenyl moiety having attached a 6 membered partially saturated or unsaturated ring such as a C<sub>6</sub> cycloalkenyl, i.e. hexene, or a C<sub>5</sub> cycloalkenyl moiety, such as cyclopentene.

Preferred compounds useful in the present invention include:

(Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-  
15 bicyclo[3.2.1]octane iodide;  
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile;  
(Endo)-8-methyl-3-(2,2,2-triphenyl-ethyl)-8-aza-bicyclo[3.2.1]octane;  
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide;  
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionic acid;  
20 (Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
iodide;  
(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
bromide;  
3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propan-1-ol;  
25 *N*-Benzyl-3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-  
propionamide;  
(Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
iodide;  
1-Benzyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-  
30 urea;  
1-Ethyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;  
*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-acetamide;

- N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide;  
 3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile;  
 (Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-  
 bicyclo[3.2.1]octane iodide;
- 5 *N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-  
 benzenesulfonamide;  
 [3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;  
*N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-  
 methanesulfonamide; and
- 10 (Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-  
 azonia-bicyclo[3.2.1]octane bromide.
- More preferred compounds useful in the present invention include:  
 (Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-  
 bicyclo[3.2.1]octane iodide;
- 15 (Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
 iodide;  
 (Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
 bromide;  
 (Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane  
 20 iodide;  
 (Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-  
 bicyclo[3.2.1]octane iodide; and  
 (Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-  
 azonia-bicyclo[3.2.1]octane bromide.
- 25

### **Methods of Preparation.**

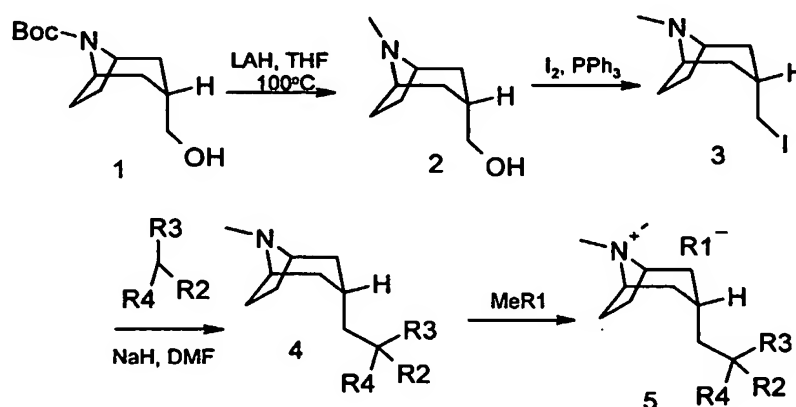
#### **Preparation**

- The compounds of Formula (I) and Formula (II) may be obtained by applying  
 synthetic procedures, some of which are illustrated in the Schemes below. The  
 30 synthesis provided for these Schemes is applicable for producing compounds of  
 Formula (I) and Formula (II) having a variety of different R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> which are

reacted, employing substituents which are suitable protected, to achieve compatibility with the reactions outlined herein. Subsequent deprotection, in those cases, then affords compounds of the nature generally disclosed. While some schemes are shown with compounds only of Formula (II), this is merely for illustration purpose only.

- 5 The general preparation method is shown in **Scheme I**. The synthesis started with compound **1**. Reduction with lithium aluminium hydride (LAH) afforded alcohol **2**. Displacement with iodine provided **3**. Coupling reaction with the anion derived from  $\text{HCR}_2(\text{R}_3)(\text{R}_4)$  then furnished compound **4**, which was easily converted to ammonium salt **5**.

10 **Scheme I.**

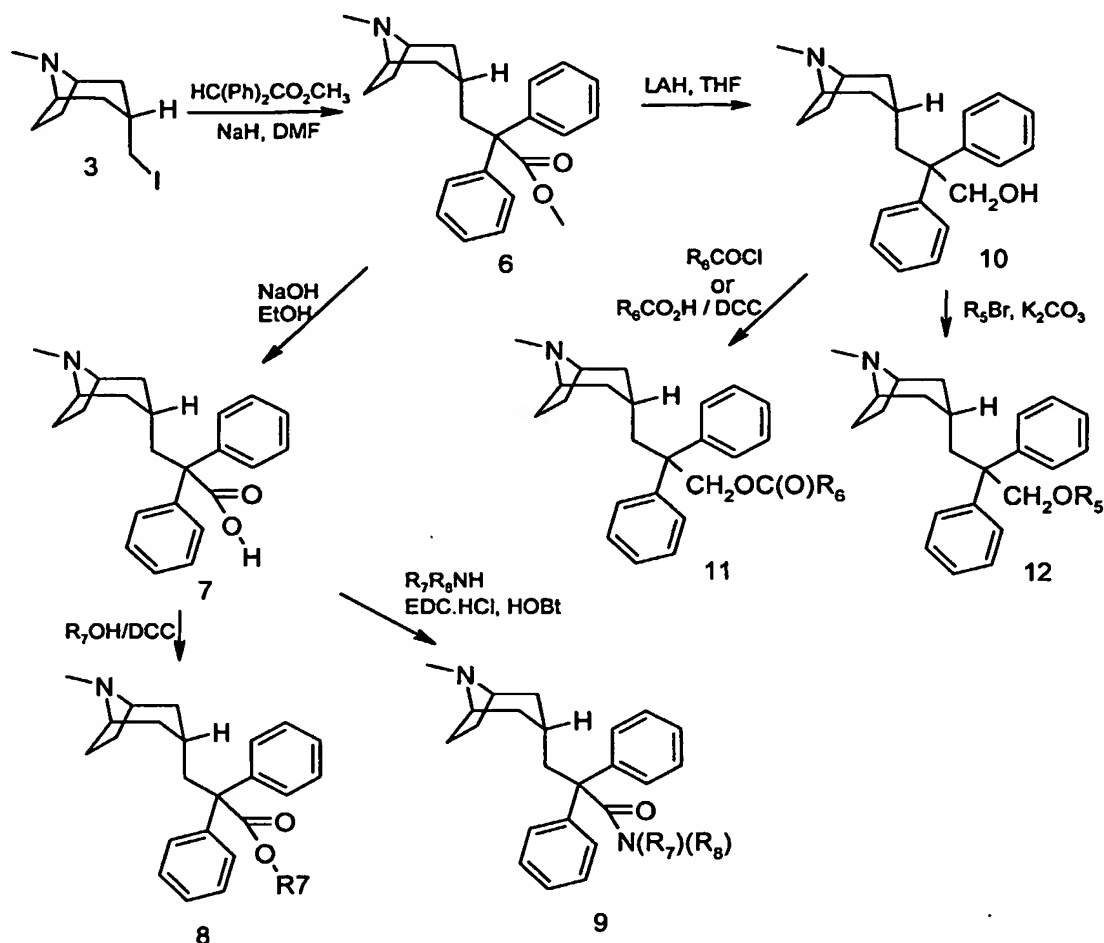


- A more specific preparation method leading to compounds with Formula (II) is outlined in **Scheme II**. Alkylation of ester  $\text{HC}(\text{Ph})_2\text{CO}_2\text{CH}_3$  with **3** afforded compound **6**. Hydrolysis of **6** generated acid **7**. 1,3-Dicyclohexylcarbodiimide (DCC) mediated condensation of the acid with alcohol  $(\text{R}_7)\text{OH}$  then furnished ester **8**. Condensation of acid **7** with amine  $(\text{R}_7)(\text{R}_8)\text{NH}$  under suitable amide coupling conditions well known to those skilled in the art such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) and 1-hydroxybenzotriazole hydrate (HOBt) provided amide **9**. Reduction of **6** generated alcohol **10**. Reaction of **10** with acid chloride  $(\text{R}_6)\text{COCl}$  or acid  $(\text{R}_6)\text{CO}_2\text{H}$  afforded ester **11**. Alkylation of **10** with appropriate reagents such as  $(\text{R}_5)\text{Br}$  then furnished **12**.

Compounds with structures similar to **6**, **7**, **8**, **9**, **10**, **11** and **12** were converted to corresponding ammonium salts by reacting with appropriate reaction reagents such as

MeBr and MeI (not shown in the scheme). Appropriate protection and deprotection methods were utilized in some preparation procedures.

**Scheme II.**



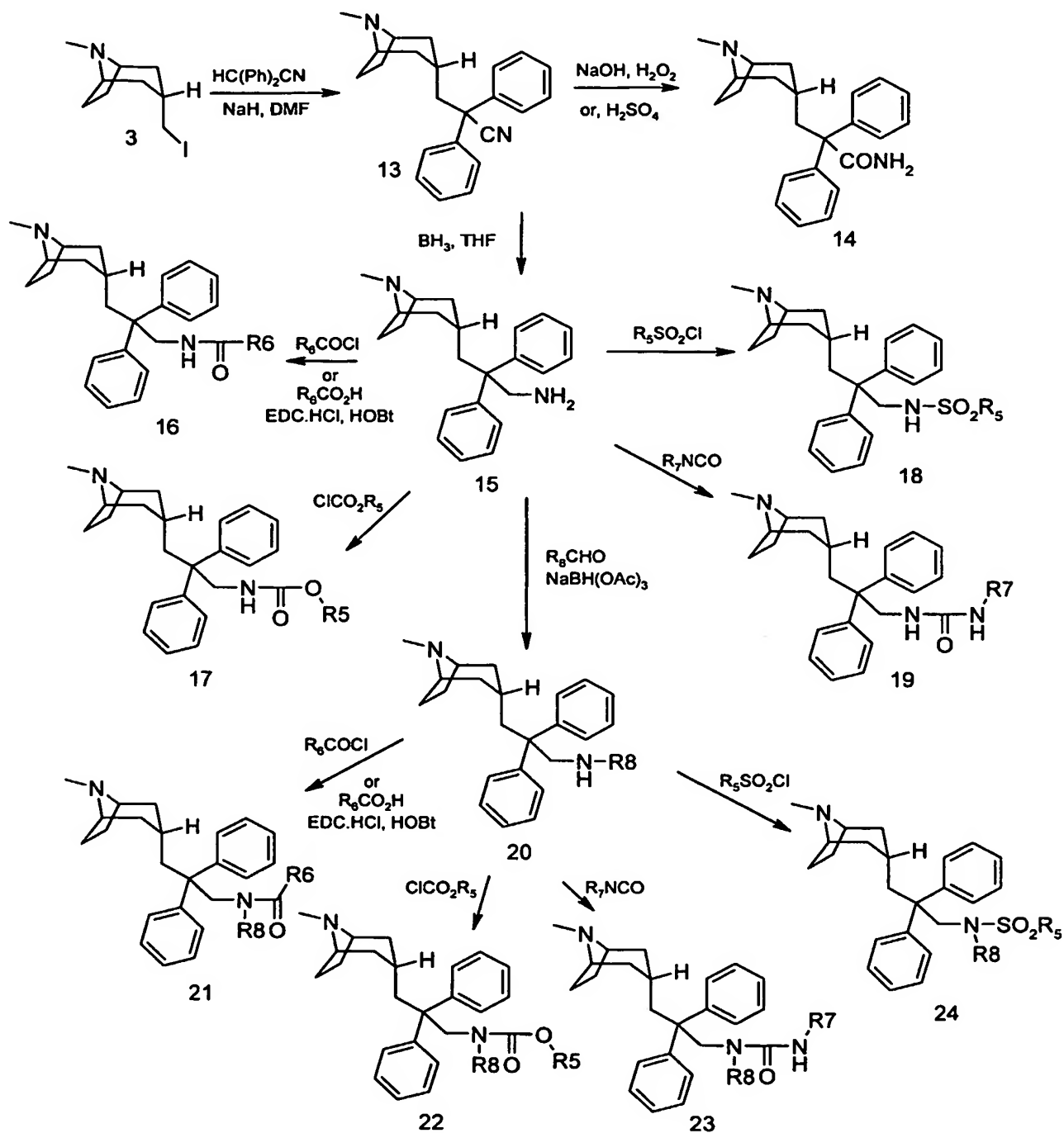
- 5 A more specific preparation method leading to compounds with Formula (II) is outlined in **Scheme III**. Alkylation of  $\text{HC(Ph)}_2\text{CN}$  with 3 provided nitrile 13. Hydrolysis of 13 under either basic conditions (e.g.,  $\text{NaOH}$  and  $\text{H}_2\text{O}_2$ ) or acidic conditions (e.g.,  $\text{H}_2\text{SO}_4$ ) afforded amide 14. Reduction of 13 led to amine 15 that was conveniently transformed to amide 16, carbamide 17, sulfonamide 18 and urea 19.
- 10 Condensation of 15 with aldehyde  $(\text{R}_8)\text{CH(O)}$  followed by reduction with  $\text{NaBH(OAc)}_3$  furnished amine 20 that was easily converted to amide 21, carbamide 22, urea 23 and sulfonamide 24.

Compounds with structures similar to 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24 were converted to corresponding ammonium salts by reacting with appropriate



reaction reagents such as MeBr and MeI (not shown in the scheme). Appropriate protection and deprotection methods were utilized in some preparation procedures.

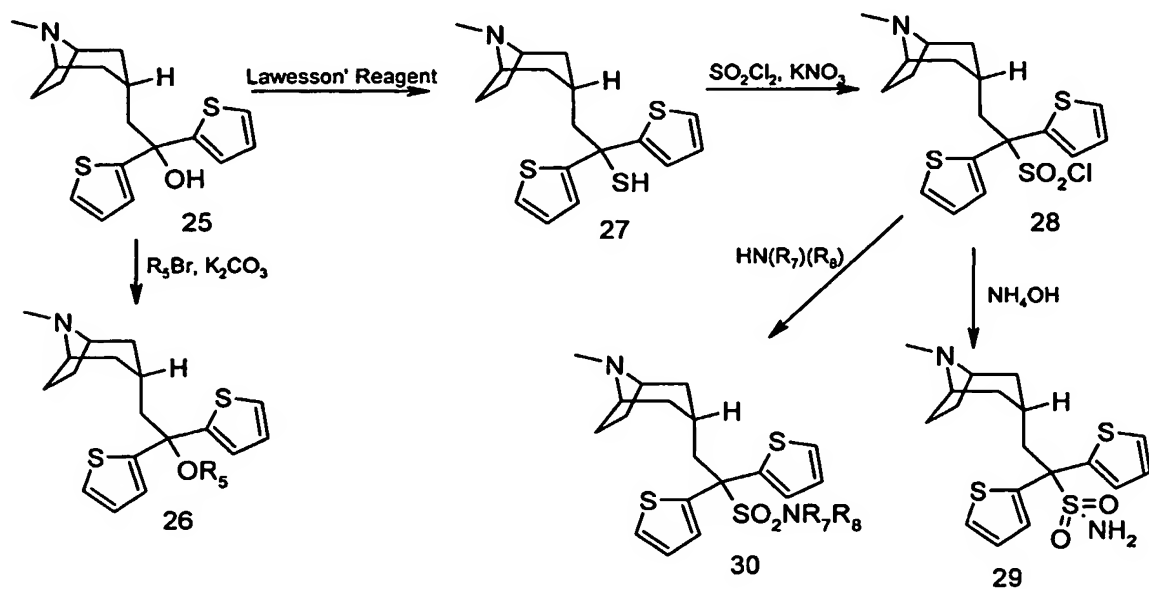
**Scheme III.**



A more specific preparation method leading to compounds with Formula (II) is outlined in **Scheme IV**. Alkylation of **25** with (R<sub>5</sub>)Br provided **26**. Reaction of **25** with Lawesson's reagent afforded **27**. Oxidation of **27** with SO<sub>2</sub>Cl<sub>2</sub> and KNO<sub>3</sub> furnished **28** that was converted to either **29** or sulfonamide **30**.

5 Compounds with structures similar to **26**, **27**, **29** and **30** were easily converted to the corresponding ammonium salts by reacting with appropriate reaction reagents such as MeBr and MeI (not shown in the scheme). Appropriate protection and deprotection methods were utilized in some preparation procedures.

**Scheme IV**



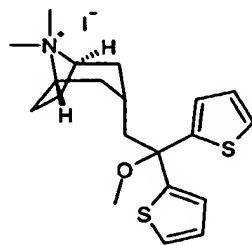
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### SYNTHETIC EXAMPLES

The following examples are provided as illustrative of the present invention but not limiting in any way:

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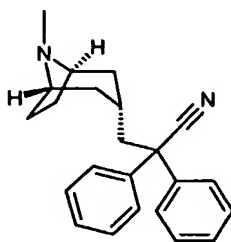
#### Example 1



(Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide

To a solution of 2-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1,1-dithiophen-2-yl-ethanol (prepared according to US2800482) (212mg, 0.64mmol) in 5 mL methylenechloride and iodomethane (0.40mL, 6.4 mmol), 50% aqueous potassium hydroxide (0.25mL, 3.2 mmol) and tetrabutylammonium chloride (5mg, 3 mol%) was added. The reaction mixture was heated to reflux for 5 d. Each day, an additional 0.2mL iodomethane and 0.1mL potassium hydroxide was added. Upon completion, the reaction mixture was cooled to room temperature, diluted with methylenechloride and washed with water. The aqueous layer was extracted with methylenechloride and the combined organic layers were washed with brine, dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude product was recrystallized from methylenechloride/ethyl acetate to give 109mg of the title compound: LCMS (ES)  $m/z$  362 (M)<sup>+</sup>.

Example 2



3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile

2a) Preparation of ((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-methanol

A mixture of 1,1-dimethylethyl (endo)-3-(hydroxymethyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (0.50 g, 2.05 mmol) and  $\text{LiAlH}_4$  (6.16 mL, 1.0 M in THF, 6.16 mmol) was heated at 80°C with a microwave reactor for 60 min. The solution was then mixed with saturated  $\text{Na}_2\text{SO}_4$  solution, filtered through celite and concentrated to afford the title compound (0.31 g, 97 %): LCMS (ES)  $m/z$  156 (M+H)<sup>+</sup>;  $^1\text{H}$ -NMR( $\text{CDCl}_3$ )  $\delta$  1.28 (s, 1H), 1.59 (m, 4H), 1.90 (m, 1H), 2.13 (m, 4H), 2.32 (s, 3H), 3.17 (s, 2H), 3.59 (d, 2H).

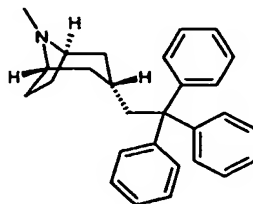
2b) Preparation of (endo)-3-iodomethyl-8-methyl-8-aza-bicyclo[3.2.1]octane

A solution of iodine (6.67 g, 25.8 mmol) and ((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-methanol (2.0 g, 12.9 mmol) in  $\text{CH}_2\text{Cl}_2$  (120 mL) was mixed with  $\text{PPh}_3$  (on resin, 8.6 g, 3 mmol/g, 25.8 mmol). The resultant mixture was stirred for 17 hours, filtered and concentrated to afford the title compound (2.63 g, 77 %): LCMS (ES)  $m/z$  266 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{CDCl}_3)$   $\delta$  2.05 (m, 4H), 2.39 (m, 3H), 2.79 (d, 3H), 2.98 (m, 2H), 3.45 (d, 2H), 3.81 (s, 2H).

2c) Preparation of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile

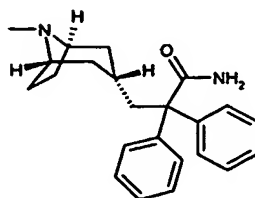
A solution of (endo)-3-iodomethyl-8-methyl-8-aza-bicyclo[3.2.1]octane (1.06 g, 4.0 mmol) and  $\text{Ph}_2\text{CHCN}$  (2.32 g, 12.0 mmol) in DMF (20 mL) was mixed with NaH (0.288 g, 12.0 mmol). The resultant mixture was stirred at room temperature for 60 minutes. Filtration and purification via a reverse phase HPLC (Gilson) then afforded the title compound (1.16 g, 93 %): LCMS (ES)  $m/z$  331 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{CDCl}_3)$   $\delta$  1.64 (m, 2H), 2.14 (m, 1H), 2.26 (m, 2H), 2.34 (m, 2H), 2.52 (m, 2H), 2.75 (m, 5H), 3.83 (s, 2H), 7.39 (d, 10H).

### Example 3

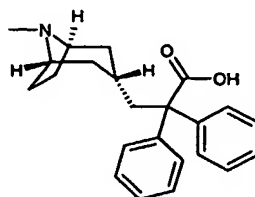


#### (Endo)-8-methyl-3-(2,2,2-triphenyl-ethyl)-8-aza-bicyclo[3.2.1]octane

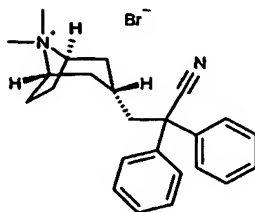
A solution of triphenylmethane (0.276 g, 1.13 mmol) in THF (0.5 mL) was mixed with  $n\text{-BuLi}$  (0.706 mL, 1.6 M in Hexane, 1.13 mmol). The solution was stirred for 10 minutes and added by a solution of (endo)-3-iodomethyl-8-methyl-8-aza-bicyclo[3.2.1]octane (100 mg, 0.377 mmol) in DMF (1.0 mL). The mixture was stirred at room temperature for 60 minutes, mixed with  $\text{H}_2\text{O}$  (0.1 mL), concentrated and filtered. Purification via a reverse phase HPLC (Gilson) then afforded the title compound (23.8 mg, 17 %): LCMS (ES)  $m/z$  382 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{CDCl}_3)$   $\delta$  1.07 (d, 2H), 2.12 (m, 1H), 2.22 (m, 4H), 2.31 (m, 2H), 2.65 (d, 3H), 2.97 (d, 2H), 3.63 (s, 2H), 7.21 (m, 3H), 7.30 (d, 12H).

**Example 4****3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide**

A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile (53 mg, 0.16 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.25 mL) was mixed with H<sub>2</sub>SO<sub>4</sub> (0.28 mL, 96 %) and stirred at 40°C for 30 hours. The mixture was then poured into ice, neutralized with NH<sub>3</sub>.H<sub>2</sub>O, extracted with EtOAc and concentrated. The resultant residue was dissolved in DMSO and filtered. Purification via a reverse phase HPLC (Gilson) provided the title compound (17.2 mg, 30 %): MS (ES) *m/z* 347 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δ 1.31 (d, 2H), 1.98 (m, 1H), 2.28 (m, 4H), 2.39 (m, 2H), 2.67 (d, 3H), 2.79 (d, 2H), 3.66 (s, 2H), 5.82 (s, br, 1H), 6.88 (s, br, 1H), 7.37 (m, 10H).

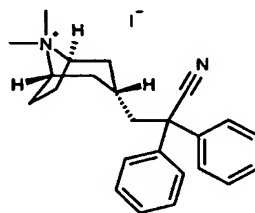
**Example 5****3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionic acid**

A solution of 2-[(3-endo)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl]-1,1-diphenylethanol (100 mg, 1.56 mmol) in HCOOH (0.25 mL) was quickly added by H<sub>2</sub>SO<sub>4</sub> (2.73 mL, 90 %) at 0°C. The reaction vial was capped immediately and stored in a refrigerator at -20°C for 7 days. The solution was poured into ice, neutralized with NH<sub>3</sub>.H<sub>2</sub>O, extracted with EtOAc and concentrated. The resultant residue was dissolved in DMSO and filtered. Purification via a reverse phase HPLC (Gilson) then afforded the title compound (52 mg, 48 %): LCMS (ES) *m/z* 350 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.39 (d, 2H), 1.86 (m, 1H), 1.97 (m, 2H), 2.30 (m, 4H), 2.69 (s, 3H), 2.84 (d, 2H), 3.69 (s, 2H), 7.28 (m, 2H), 7.36 (m, 8H).

**Example 6**

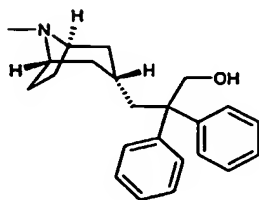
**(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide**

5           A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile (310 mg, 0.938 mmol) in acetone (6.0 mL) was mixed with MeBr (4.69 mL, 2.0 M in *t*-BuOMe, 9.38 mmol). The resultant mixture was stirred at room temperature for 60 minutes and filtered. The solid was washed with acetone (2 x 3 mL) to afford the title compound (333 mg, 83 %): LCMS (ES) *m/z* 345 (*M*)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.82 (d, 2H), 2.17 (m, 1H), 2.35 (m, 2H), 2.49 (m, 4H), 3.01 (d, 2H),  
10       3.07 (s, 3H), 3.10 (s, 3H), 3.79 (s, 2H), 7.36 (m, 2H), 7.43 (m, 4H), 7.49 (m, 4H).

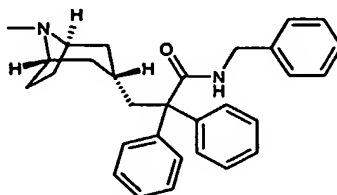
**Example 7**

**(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide**

15           A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile (26.5 mg, 0.080 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) and MeCN (0.5 mL) was mixed with MeI (0.125 mL, 2.00 mmol). The resultant mixture was stirred at room temperature for 3 hours, diluted with DMSO (0.3 mL) and concentrated. Purification  
20       via a reverse phase HPLC (Gilson) then afforded the title compound (22.9 mg, 60 %): LCMS (ES) *m/z* 345 (*M*)<sup>+</sup>; <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δ 1.83 (d, 2H), 2.17 (m, 1H), 2.35 (m, 2H), 2.49 (m, 4H), 3.01 (d, 2H), 3.07 (s, 3H), 3.10 (s, 3H), 3.79 (s, 2H), 7.36 (m, 2H), 7.43 (m, 4H), 7.49 (m, 4H).

**Example 8****3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propan-1-ol**

A mixture of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-  
5 propionic acid (42.5 mg, 0.122 mmol) and  $\text{LiAlH}_4$  (0.488 mL, 1.0 M in THF, 0.488  
mmol) was heated with a microwave reactor at 100°C for 1 hour. It was diluted with  
saturated  $\text{Na}_2\text{SO}_4$  solution, filtered through celite and concentrated. The resultant  
residue was dissolved in DMSO and filtered. Purification via a reverse phase HPLC  
(Gilson) then afforded the title compound (29.1 mg, 71 %): LCMS (ES)  $m/z$   
10 336(M+H)<sup>+</sup>; <sup>1</sup>H-NMR( $\text{CDCl}_3$ )  $\delta$  1.40 (d, 2H), 1.92 (m, 1H), 2.29 (m, 6H), 2.59 (m,  
2H), 2.68 (d, 3H), 3.72 (s, 2H), 4.16 (s, 2H), 7.13 (m, 3H), 7.30 (m, 7H).

**Example 9**

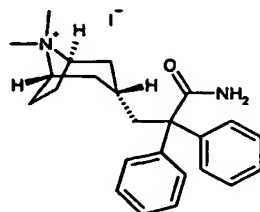
15

**N-Benzyl-3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide**

A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-  
propionic acid (82.0 mg, 0.235 mmol) in  $\text{CH}_2\text{Cl}_2$  (3.0 mL) was mixed with  $\text{PhCH}_2\text{NH}_2$   
20 (28.2  $\mu\text{L}$ , 0.258 mmol), EDC (49.5 mg, 0.258 mmol), HOBT (3.2 mg, 0.024 mmol) and  
( $\text{CH}_3\text{CH}_2$ )<sub>3</sub>N (0.232 mL, 1.65 mmol). The mixture was stirred at room temperature for  
60 hours and concentrated. The resultant residue was dissolved in DMSO and filtered.  
Purification via a reverse phase HPLC (Gilson) then afforded the title compound (29.8  
mg, 30 %): LCMS (ES)  $m/z$  439(M+H)<sup>+</sup>; <sup>1</sup>H-NMR( $\text{CDCl}_3$ )  $\delta$  1.34 (d, 2H), 1.96 (m,

1H), 2.23 (m, 2H), 2.38 (m, 4H), 2.63 (d, 3H), 2.83 (d, 2H), 3.66 (s, 2H), 4.41 (d, 2H), 6.93 (m, 2H), 7.22 (m, 3H), 7.38 (m, 10H).

### Example 10

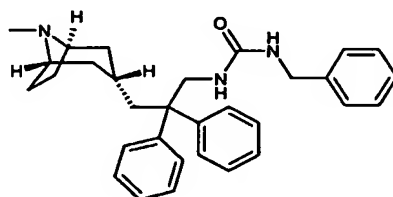


5

#### (Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide

The title compound was prepared from 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide by following the procedure of Example 7 (33 %  
 10 yield): LCMS (ES)  $m/z$  363 ( $M^+$ );  $^1\text{H-NMR}(\text{CDCl}_3)$   $\delta$  1.49 (d, 2H), 1.95 (m, 1H), 2.25 (m, 2H), 2.42 (m, 4H), 2.84 (d, 2H), 3.17 (s, 3H), 3.23 (s, 3H), 3.93 (s, 2H), 5.65 (s, 1H), 5.91 (s, 1H), 7.39 (m, 10H).

### Example 11



15

#### 1-Benzyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea

##### 11a) 3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine

A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile (250 mg, 0.758 mmol) in THF (2.5 mL) was mixed with  $\text{BH}_3$  (2.53 mL, 1.5 M in THF, 3.79 mmol) at  $0^\circ\text{C}$ . The mixture was stirred at room temperature for 20  
 20 hours and diluted with  $\text{H}_2\text{O}$  (1.0 mL). The solution was then mixed with  $\text{K}_2\text{CO}_3$  (0.1 g) and stirred at room temperature for 1 hour. Organic layers were separated and the aqueous part was extracted with EtOAc (2 x 3 mL). The organic layers were



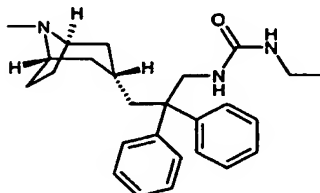
combined, dried over  $\text{Na}_2\text{SO}_4$  and concentrated. Purification via a reverse phase HPLC (Gilson) afforded the titled compound (159mg, 63%): LCMS (ES)  $m/z$  335 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{MeOD})$   $\delta$  1.35 (d, 2H), 2.01 (m, 3H), 2.34 (s, 4H), 2.55 (s, 2H), 2.68 (s, 3H), 3.73 (m, 5H), 7.26 (m, 4H), 7.33 (m, 2H), 7.43 (m, 4H).

5 11b) 1-Benzyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea

A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine (50.0 mg, 0.149 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.0 mL) was mixed with  $\text{PhCH}_2\text{NCO}$  (20.4  $\mu\text{L}$ , 0.164 mmol) and  $(\text{CH}_3\text{CH}_2)_3\text{N}$  (62.8  $\mu\text{L}$ , 0.447 mmol). The result mixture  
10 was stirred at room temperature for 1 hours and concentrated. Purification via a reverse phase HPLC (Gilson) then afforded the titled compound (13.0mg, 19%): LCMS (ES)  $m/z$  468 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{MeOD})$   $\delta$  1.24 (d, 2H), 1.94 (m, 3H), 2.25 (m, 4H), 2.49 (d, 2H), 2.67 (s, 3H), 3.62 (s, 2H), 3.97 (s, 2H), 4.23 (s, 2H), 7.22 (m, 6H), 7.33 (m, 4H).

15

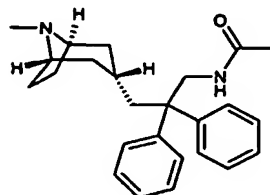
Example 12



1-Ethyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea

The title compound was prepared from 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine and  $\text{CH}_3\text{CH}_2\text{NCO}$  by following the  
20 procedure in Example 11 (45% yield): LCMS (ES)  $m/z$  406 ( $\text{M}+\text{H}^+$ );  $^1\text{H-NMR}(\text{MeOD})$   $\delta$  1.03 (t, 3H), 1.33 (d, 2H), 1.94 (m, 3H), 2.25 (m, 4H), 2.55 (d, 2H), 2.67 (s, 3H), 3.07 (q, 2H), 3.68 (s, 2H), 3.94 (s, 2H), 7.24 (m, 6H), 7.34 (m, 4H).

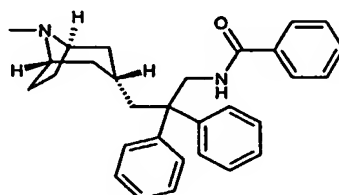
Example 13



N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-acetamide

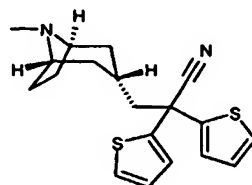
A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine (33.4 mg, 0.10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was mixed with Ac<sub>2</sub>O (18.9  $\mu$ L, 0.20 mmol) and pyridine (16.2  $\mu$ L, 0.20 mmol). The mixture was stirred at room temperature for 1 hour and concentrated. Purification *via* a reverse phase HPLC (Gilson) then afforded the title compound (10.7mg, 29%): LCMS (ES) *m/z* 377 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD)  $\delta$  1.26 (d, 2H), 1.82 (s, 3H), 1.96 (m, 3H), 2.26 (s, 4H), 2.53 (d, 2H), 2.67 (s, 3H), 3.66 (s, 2H), 4.00 (s, 2H), 7.24 (m, 6H), 7.33 (m, 4H).

10

Example 14N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide

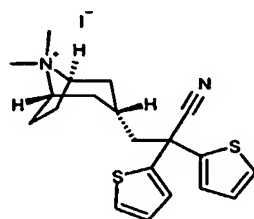
The title compound was prepared from 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine and (PhCO)<sub>2</sub>O by following the procedure in Example 13 (8% yield): LCMS (ES) *m/z* 439 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD)  $\delta$  1.28 (d, 2H), 2.00 (m, 3H), 2.24 (s, 4H), 2.59 (d, 2H), 2.67 (s, 3H), 3.65 (s, 2H), 4.21 (s, 2H), 7.31 (m, 6H), 7.39 (m, 6H), 7.50 (m, 3H).

20

Example 153-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile

The title compound was prepared from (endo)-3-iodomethyl-8-methyl-8-aza-bicyclo[3.2.1]octane and 2,2-di-thiophen-2-yl-acetonitrile by following the procedure in Example 2C (34 % yield): LCMS (ES)  $m/z$  343 ( $M+H$ )<sup>+</sup>; <sup>1</sup>H-NMR(CDCl<sub>3</sub>)  $\delta$  1.79 (m, 2H), 2.21 (m, 2H), 2.33 (m, 2H), 2.62 (m, 2H), 2.73 (m, 4H), 3.80 (m, 2H), 4.35 (s, 2H), 7.02 (m, 2H), 7.23 (m, 2H), 7.37 (m, 2H).

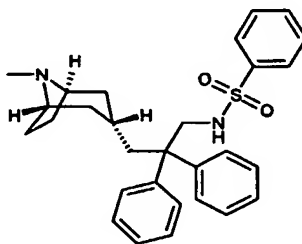
### Example 16



(Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane iodide

The title compound was prepared from 3-((endo)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile by following the procedure in Example 7 (43 %): LCMS (ES)  $m/z$  345 ( $M$ )<sup>+</sup>; <sup>1</sup>H-NMR(CDCl<sub>3</sub>)  $\delta$  1.82 (d, 2H), 2.35 (m, 2H), 2.23 (m, 3H), 2.58 (m, 4H), 2.82 (m, 2H), 3.37 (s, 6H), 4.25 (s, 2H), 7.02 (m, 2H), 7.24 (m, 2H), 7.36 (m, 2H).

### Example 17

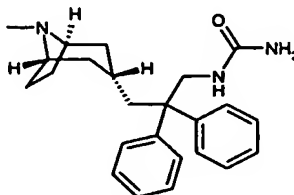


N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzenesulfonamide

A solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine (67.0 mg, 0.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) was mixed with PhSO<sub>2</sub>Cl (28.2  $\mu$ L, 0.22 mmol) and (CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>N (84.3  $\mu$ L, 0.60 mmol). The result mixture was stirred at room temperature for 1 hours and concentrated. Purification *via* a reverse phase HPLC (Gilson) then afforded the title compound (51.5mg, 54%): LCMS (ES)  $m/z$  475

(M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.39 (d, 2H), 2.01 (m, 3H), 2.30 (s, 4H), 2.69 (s, 5H), 3.60 (s, 2H), 3.68 (s, 2H), 7.12 (m, 4H), 7.27 (m, 6H), 7.55 (m, 2H), 7.63(m, 1H), 7.78 (m, 2H).

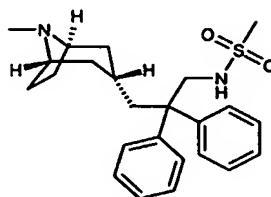
### Example 18



#### [3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea

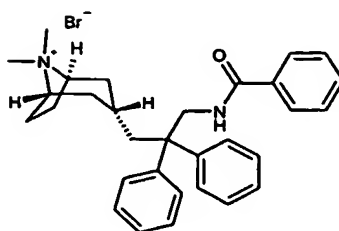
To a solution of 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine (50.0 mg, 0.149 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4.0 mL), ClSO<sub>2</sub>NCO (31.2 μL, 0.358 mmol) was added. The mixture was stirred at room temperature for 2 days and concentrated. Purification via a reverse phase HPLC (Gilson) then afforded the title compound (21.6mg, 38%): LCMS (ES) *m/z* 378 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.33 (d, 2H), 2.01 (m, 3H), 2.29 (s, 4H), 2.57 (m, 2H), 2.68 (s, 3H), 3.69 (s, 2H), 4.01 (s, 2H), 7.25 (m, 6H), 7.34 (m, 4H).

### Example 19



#### N-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-methanesulfonamide

The title compound was prepared from 3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propylamine and MeSO<sub>2</sub>Cl by following the procedure in Example 17 (28% yield): LCMS (ES) *m/z* 413 (M+H)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.39 (d, 2H), 1.97 (m, 3H), 2.30 (s, 4H), 2.68 (s, 3H), 2.76 (s, 3H), 3.68 (s, 2H), 3.84 (s, 2H), 7.23 (s, 6H), 7.33 (s, 4H).

**Example 20**

**(Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide**

- 5           A solution of *N*-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide (29 mg, 0.0683 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) and acetone (0.5 mL) was mixed with MeBr (0.342 mL, 2.0 M in *t*-butyl methyl ether, 0.683 mmol). The resultant mixture was stirred at room temperature for 3 hours and concentrated. Purification via a reverse phase HPLC (Gilson) then afforded the title compound (19.6
- 10 mg, 64 %): LCMS (ES) *m/z* 453 (M)<sup>+</sup>; <sup>1</sup>H-NMR(MeOD) δ 1.20 (d, 2H), 2.32 (m, 7H), 2.65 (d, 2H), 2.98 (s, 3H), 3.02 (s, 3H), 3.60 (s, 2H), 4.22 (s, 2H), 7.30(m, 6H), 7.39(m, 6H), 7.50 (s, 3H).

**BIOLOGICAL EXAMPLES**

15

The inhibitory effects of compounds at the M<sub>3</sub> mAChR of the present invention are determined by the following *in vitro* and *in vivo* assay:

**Analysis of Inhibition of Receptor Activation by Calcium Mobilization:**

- 20 Stimulation of mAChRs expressed on CHO cells were analyzed by monitoring receptor-activated calcium mobilization as previously described<sup>10</sup>. CHO cells stably expressing M<sub>3</sub> mAChRs were plated in 96 well black wall/clear bottom plates. After 18 to 24 hours, media was aspirated and replaced with 100 µl of load media (EMEM with Earl's salts, 0.1% RIA-grade BSA (Sigma, St. Louis MO), and 4 µM Fluo-3-
- 25 acetoxymethyl ester fluorescent indicator dye (Fluo-3 AM, Molecular Probes, Eugene, OR) and incubated 1 hr at 37° C. The dye-containing media was then aspirated, replaced with fresh media (without Fluo-3 AM), and cells were incubated for 10

minutes at 37° C. Cells were then washed 3 times and incubated for 10 minutes at 37° C in 100 µl of assay buffer (0.1% gelatin (Sigma), 120 mM NaCl, 4.6 mM KCl, 1 mM KH<sub>2</sub> PO<sub>4</sub>, 25 mM NaH CO<sub>3</sub>, 1.0 mM CaCl<sub>2</sub>, 1.1 mM MgCl<sub>2</sub>, 11 mM glucose, 20mM HEPES (pH 7.4)). 50 µl of compound ( $1 \times 10^{-11}$  –  $1 \times 10^{-5}$  M final in the assay) was  
5 added and the plates were incubated for 10 min. at 37° C. Plates were then placed into a fluorescent light intensity plate reader (FLIPR, Molecular Probes) where the dye loaded cells were exposed to excitation light (488 nm) from a 6 watt argon laser. Cells were activated by adding 50 µl of acetylcholine (0.1-10 nM final), prepared in buffer containing 0.1% BSA, at a rate of 50 µl/sec. Calcium mobilization, monitored as  
10 change in cytosolic calcium concentration, was measured as change in 566 nm emission intensity. The change in emission intensity is directly related to cytosolic calcium levels<sup>11</sup>. The emitted fluorescence from all 96 wells is measured simultaneously using a cooled CCD camera. Data points are collected every second. This data was then plotting and analyzed using GraphPad PRISM software.

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#### **Methacholine-induced bronchoconstriction**

Airway responsiveness to methacholine was determined in awake, unrestrained BalbC mice ( $n = 6$  each group). Barometric plethysmography was used to measure enhanced pause (Penh), a unitless measure that has been shown to correlate with the changes in  
20 airway resistance that occur during bronchial challenge with methacholine<sup>12</sup>. Mice were pretreated with 50 µl of compound (0.003-10 µg/mouse) in 50 µl of vehicle (10% DMSO) intranasally, and were then placed in the plethysmography chamber. Once in the chamber, the mice were allowed to equilibrate for 10 min before taking a baseline Penh measurement for 5 minutes. Mice were then challenged with an aerosol of  
25 methacholine (10 mg/ml) for 2 minutes. Penh was recorded continuously for 7 min starting at the inception of the methacholine aerosol, and continuing for 5 minutes afterward. Data for each mouse were analyzed and plotted by using GraphPad PRISM software.